

The action of osmic acid does not seem to sufficiently harden the walls and cell-contents of the most delicate Florideæ (Callithamnion, Griffithsia, fringing hairs of Spyridias, Dasya, etc.) to prevent their shrinking even in very dilute glycerine, but no one of many other reagents experimented with gave much better results. Perhaps some of the workers at Wood's Holl, the present season, can remove the difficulty.

Most fungi suffer no change in dilute glycerine, although not previously hardened, and they may be well preserved in glycerine jelly. Such as are too delicate to do so otherwise may be enabled, in most cases, to withstand the distorting influence of glycerine by hardening in osmic acid, as described for the algæ. I have not yet succeeded, however, in satisfactorily preserving Saprolegniaceæ in this way, though the most delicate Mucoraceæ and Hyphomycetes do finely.

In short, it is not too much to say that the way is opened, by the process above described, toward the abandonment of fluids and cements and all the bothersome manipulation connected with their use, and the substitution of a technique simpler in detail and far more satisfactory in results.

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On the nature of certain plant diseases.

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De Bary in his paper "On some Sclerotiniæ and sclerotium diseases,"¹ published in 1886, was the first to show that Sclerotinia (Peziza) sclerotiorum while apparently growing as a parasite actually grows as a saprophyte, but gives off in the process of its growth a ferment which swells the cell-walls and kills the tissue of the host, thus preparing the way for the fungus. In 1888 Marshall-Ward described a Botrytis growing upon a Lilium candidum which behaves in the same manner.² De Bary found that liquid obtained from vegetable tissue infested with Sclerotinia was capable of producing the characteristic decomposition of pieces of healthy tissue placed in it. Marshall-Ward not only obtained this same result, but also observed under the microscope drops of

¹ Bot. Zeit. 1886, nos. 22, 23.

² Annals of Botany, Nov., 1888. Vol. ii, no. vii.

a glairy fluid exuding from the tips of vigorous hyphæ, and I have studied this same *Botrytis* growing upon *Lilium longiflorum* and have observed the same phenomena.

Last autumn my attention was called to a rot of the sweet potato in which *Rhizopus nigricans* (*Mucor stolonifer*) was present; yet if spores of this fungus were sown upon a slice of healthy sweet potato they produced no effect, the spores not even germinating; while if a bit of the mycelium were placed in a similar position, rapid growth of the fungus and decomposition of the potato followed. If the slice had been previously killed by boiling, the spores germinated readily, producing the same results as the bit of mycelium. *Rhizopus* behaves in the same way in cultures made upon Irish potatoes, beets, turnips, carrots, apples, pears, and quinces. De Bary remarked this peculiarity in *Sclerotinia*, and thought that it was due to the fact that the fungus required to be nourished as a saprophyte in order to become facultative as a parasite. The habit of the *Botrytis* of the lily disease is slightly different. Its spores germinate readily in a drop of distilled water if the temperature be favorable,³ but then they are large and contain considerable reserve material stored in them; while the conidia of the before-mentioned fungi are small and require external nourishment in order to be able to grow.

The following experiment illustrates this point, and also connects the *Rhizopus* definitely with the origin of the disease. A hanging drop culture of *Rhizopus* was made in a drop of sterilized orange juice, in which this fungus grows freely. When this culture was well under way the coverslip from which the drop depended was inverted and placed in the middle of a slice of healthy sweet potato. This was left over night, and in the morning it was found that the hyphæ were reaching over the edge of the coverslip and producing decay wherever they touched.

As De Bary found with *Sclerotinia* that the liquid squeezed from diseased portions of the host was poisonous to healthy tissue, so I have found with *Rhizopus*. The liquid was squeezed from a thoroughly rotten potato and filtered. Slices of healthy vegetable tissue immersed in this fluid were soon softened and decomposed. Other experiments were made, filtering the liquid through a porcelain filter, and (to entirely eliminate the chance of bacterial action) adding a drop of corrosive sublimate to each saucer of the liquid. The

³ Kean, A Lily Disease in Bermuda. BOT. GAZETTE, Jan., 1890.

result was the same as that obtained in the previous experiments. Some efforts were made at obtaining the ferment in a pure state with a measure of success. Marshall-Ward found that alcohol coagulated the ferment. I found that on adding 20 cc. of 90 per cent. alcohol to 10 cc of the filtered liquid obtained as in the former experiments, a heavy gray flocculent precipitate was formed. This was filtered off and the filtrate was evaporated at a low temperature. Its residue was then redissolved in water. Pieces of vegetable tissue put into this liquid underwent no change, so that it was evident that the precipitate contained the effective substance. The precipitate is readily soluble in water, and the solution thus made acts in the same way on plant tissue as the original liquid. All attempts at obtaining the ferment in a purer state have failed. It is a neutral substance, its efficiency is destroyed by heat, and seen under the microscope it is amorphous, never so far as I have seen assuming a crystalline form. Not only was this precipitate obtained directly from diseased sweet potatoes, but pure cultures of *Rhizopus* were made in flasks with sterilized orange and lemon juice as a medium. After several weeks the culture medium was decanted off, filtered and treated with alcohol. A flocculent gray precipitate was immediately formed which was similar in appearance to that obtained directly from the sweet potato, and proved to have the same destructive properties.

I have been able to get a similar, if not identical, substance, not only from the lily *Botrytis*, but also from another *Botrytis* growing upon the common live-forever (*Sedum telephium*). Besides which I have found that if leaves of potato infested with *Phytophthora infestans* (*Peronospora infestans*) be crushed in a mortar with a little water, and the liquid poured off and treated with alcohol, a similar precipitate is formed having similar properties. In the cases of *Rhizopus*, the lily *Botrytis*, and the *Botrytis* on *Sedum*, I think I have been able to show conclusively that the facultative disease agent is a chemical one. Marshall-Ward has shown it to be true of the lily *Botrytis*, while De Bary before him showed it to be so in *Sclerotinia*. From analogy, having obtained a similar ferment from *Phytophthora*, I think it but natural to conclude that here again we have another case of this method of growth.

These fungi can not be called true parasites for their manner of growing is very distinctly saprophytic, the fer-

ment killing the tissue and thus preparing the way for the growth of the fungus.

De Bary was of the opinion that fungi living thus were in an intermediate stage between parasites and saprophytes, and were gradually changing their method of growth from one to the other. However this may be, it seems to me that as these fungi do not materially differ in other respects from numbers of other fungi, we may expect to find that this method of growth is far from uncommon.

It is a well established fact that one group of fungi, bacteria, grow in this way and thus produce the so-called germ diseases. Thus it would seem that these fungous diseases, if not fungous diseases in general, are essentially the same in nature as the bacterial diseases. The production of a chemical poison in these fungi may either be simply a product of their growth, or may be a special adaptation of these organisms for obtaining food. I should incline to the former view as the more probable one. On the chemical nature of this product, and its destructiveness to any associated organisms must depend the efficiency of the fungus as an agent of disease.

It has been my purpose in this article to indicate by a few examples taken as representatives, that what has been shown to be true of one group of fungi (bacteria) as disease agents, is true of numbers of others, and that the so-called "toxic theory" of disease is capable of extension to fungous diseases in general.

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Apical growth in roots of *Marsilia quadrifolia* and *Equisetum arvense*.

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In the study of apical growth in the roots of these plants, I have found some points differing from previous accounts and perhaps worthy of note. The following will embrace these points and also the methods and sectioning employed.